REMARKS

This Amendment and Remarks are filed in response to the Office Action dated October 16, 2007 wherein claims 31-42 and 45 stand rejected. Claims 43 and 44 are withdrawn from consideration.

Double Patenting

Claims 31-42 and 45 are rejected on the ground of double patenting.

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e,g., In re Berg, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); In re Goodman, 11 F.3d 1046,29 USPQ2d 2010 (Fed. Cir. 1993); In re Longi, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); In re Van Omum, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438,164 USPQ 619 (CCPA 1970); and In re Thorington, 418 F.2d 528,163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting

ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 31-42 and 45 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 16-20 of copending Application No. 11/126,863 (published as US 2005/0249774). Although the conflicting claims are not identical, they are not patentably distinct from each other because both sets of claims are drawn to a method for treatment of migraine, migraine headache, nausea, and vomiting associated with chemotherapy, radiotherapy, surgery, pregnancy, pre-menstrual syndrome, menstruation or menopause, with the aid of an intravaginal delivery device comprising administering said intravaginal device and a composition comprising an anti-migraine or anti-nausea drug, a mucoadhesive agent, a lipophilic or hydrophilic carrier, and a sorption promoter. Many of the anti-migraine or anti-nausea drugs, mucoadhesive agents, lipophilic or hydrophilic carriers, sorption promoters and delivery devices are overlapping in scope, and some are identical (i.e. naratriptan, HPMC, saturated mono-, di-, or triglyceride of fatty acids having 8 to 18 carbons, PEG 6000/PEG 1500, ethoxydiglycol, and tampon, respectively). Therefore, the scope of the copending applications is overlapping, and thus they are obvious variants of one another.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Applicants disagree with the double patenting rejections because the claims in the 11/126,863 application are directed to the vaginal device and not to the method for treatment, however, to advance the examination Applicants submit herewith a fully executed Terminal Disclaimer disclaiming the provisionally rejected claims over the co-pending application Ser. No. 11/126,863.

Claims 31-42 and 45 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-10, 13, 15-20 and 22-23 of U.S. Patent No. 6,197,327 (hereinafter Harrison et al. '327), in view of U.S. Patent No. 6.255.502 (hereinafter Penkler et al.). Although the conflicting claims are not identical, they are not patentably distinct from each other because both sets of claims are drawn to an intravaginal drug delivery system containing an appropriate pharmaceutical agent incorporated into a pharmaceutically acceptable carrier, mucoadhesive agent, and sorption promoter, whereby the pharmaceutical agent is released into the vagina and absorbed through the vaginal mucosa. Many of the pharmaceutical agents, mucoadhesive agents, lipophilic or hydrophilic carriers, sorption promoters and delivery devices are overlapping in scope, and some are identical (i.e. ketorolac, HPMC, saturated mono-, di-, or triglyceride of fatty acids having 8 to 18 carbons, PEG 6000/PEG 1500, ethoxydiglycol, and tampon, respectively).

Harrison et al. '327 do not claim the pharmaceutical agent to comprise naratriptan. However, Penkler et 211. teach naratriptan, sumatriptan and almotriptan as suitable agents for the treatment of migraines (column 7, lines 42-47; column 10, lines 47 -48; and claims 1, 8) through vaginal administration (column 13, lines 4-5 and 8).

Therefore, it would have been prima facie obvious for one of ordinary skill in the art at the time of the instant invention to use naratriptan as the pharmaceutical agent in the vaginal delivery device of Harrison et al. '327, because Penkler et al. teach that naratriptan can be applied via the vagina for treatment of migraines.

Applicants disagree. Harrison's claims are directed to transvaginal delivery of a pharmaceutically active agent into uterus, myometrium or endometrium for treatment of dysmenorrhea rather than delivery to the systemic circulation for treatment of migraine and/or nausea, as claimed herein. However, to overcome the double patenting rejection, Applicants submit the fully Executed Terminal Disclaimer.

With submission of Terminal Disclaimers both double patenting rejections are overcome.

Rejections under 35 USC 102

Claims 31-35 are rejected under 35 U.S.C. 102(b) as being anticipated by U.S. Patent No. 6,086,909 (hereinafter Harrison et al. (909).

Harrison et al. '909 disclose a method for treatment of dysmenorrhea comprising an intravaginal drug delivery system containing an appropriate pharmaceutical agent incorporated into

a pharmaceutically acceptable carrier whereby the pharmaceutical agent is released into the vagina and absorbed through the vaginal mucosa to provide relief of dysmenorrheal (abstract). Harrison et al. '909 further disclose the pharmaceutical agent to comprise aspirin, ibuprofen, ketorolac and naproxen (column 7, lines 51-53; claim 20), and the pharmaceutically acceptable carrier comprises a hydrophilic or hydrophobic carrier, such as semi-synthetic glycerides of saturated fatty acids with 8 to 18 carbons and PEG 6000/1500, respectively (column 8, lines 8-15). Also, Harrison et al. '909 disclose the pharmaceutical formulations further comprising a mucoadhesive agent, preferably hydroxypropyl methylcellulose (column 8, lines 16-22), and a penetration enhancer, preferably ethoxydiglycol (column 8, lines 23-28). Harrison et al. '909 also disclose the method of applying the pharmaceutical formulation with the aid of an intravaginal delivery device, such as tampon device, vaginal ring, pessary, tablet, vaginal suppository, vaginal sponge, bioadhesive tablet, bioadhesive microparticle, cream, lotion, foam, ointment, solution and gel (column 2, lines 37-43; column :3, lines 8-67; column 4, lines 1-27; and column 9, line 4 through column 13, line 67). Harrison et al. '909 also disclose that preferred formulations for hydrophilic drugs comprise between about 60-90% by weight lipophilic carrier, between about 5-25% mucoadhesive agent, and between about 5-20% sorption promoter, whereas preferred formulations for lipophilic drugs comprise between about 50-90% by weight hydrophilic carrier, between about 5-20% mucoadhesive agent, and between about 5-25% sorption promoter (column 8, lines 31-34 and 44-47).

Applicants disagree. Anticipation requires that the prior art reference describes the complete invention and that both the rejected invention and the anticipating invention are the same. In this case, it is not so, particularly in view of the amended claims.

Harrison reference is directed to treatment of dysmenorrhea by delivering certain groups of analgesic and other drugs intra and transvaginally to the to vagina and through the vagina to uterus, myometrium, endometrium, and to the uterine muscle responsible for dysmenorrheal pain.

Examiner will note that anatomically, uterus, myometrium and endometrium are located in a very close vicinity of vagina. Therefore, the transport of the drugs across vaginal mucosa to the uterus in amount needed for treatment of dysmenorrheal pain is achieved primarily by delivering a needed amount of the drug to the vicinity of the vaginal mucosa, by providing means for adhering a composition comprising the drug to the vaginal mucosa and providing means for transport through the vaginal mucosa into uterus. The adhesion and transport of the drug through the vaginal mucosa directly to the targeted uterus is achieved by appropriate combination of the mucoadhesive agent and penetration enhancer present in concentrations that are appropriate for each individual drug characteristic.

The aim of the Harrison is to deliver the needed amount of the drug to uterus but limit the concentration of the drug in the systemic circulation. Systemic delivery is described by Harrison as being undesirable for treatment of dysmenorrhea.

A reason for this is that the dysmenorrheal pain originates in the uterus and the drug needs to be delivered to the uterus in an amount that eliminated or decreases the pain. This is the first and foremost distinction between the instant invention and the Harrison reference.

The origin of pain for treatment of dysmenorrhea is in the uterus that is anatomically located close to the vagina. The origin of the migraine or nausea is in the brain, anatomically very remote organ from the vagina.

As described amply in Harrison' specification, systemic administration of analgesic drug, generally by the oral route, to the patient, has not successfully relieved the conditions in many women and such administration of systemically delivered drugs leads to severe secondary symptoms due to large doses needed to achieve a relief. This failure is believed to be a result of a failure of oral administration to deliver to and achieve an efficacious dosage level of the analgesic in the uterine muscle. As described by Harrison, to achieve the efficacious levels of the drug in the uterus by systemic administration requires a very large systemic concentration of the drug in order for the needed amount to reach the targeted area, that is the uterus.

Harrison's method overcomes prior problems observed with drug delivery to the uterus, myometrium and endometrium.

On the other hand, in the instant invention, the disease or condition is such that while the systemic treatment is needed to deliver the drug to the brain to treat or prevent migraine and/or nausea, such treatment is prevented by the condition itself. Nausea, that invariably accompanies migraine, prevents oral

systemic delivery of the drugs suitable for treatment of migraine. The treated condition, i.e. nausea, thus prevents delivery of anti-migraine and anti-nausea drugs into systemic circulation, particularly so in amounts of drug needed to treat the migraine spasm or vomiting.

Contrary to the Harrison treatment where the amount in the systemic circulation is minimized or eliminated altogether, the instant method provides for large doses of the drug delivered into the systemic circulation in order to provide relief from the migraine pain or vomiting by bypassing the gastrointestinal tract.

In order to achieve this, the anti-migraine and anti-nausea drugs, claimed herein, are formulated into a composition that provides means for fast transport of the said drug directly into the systemic circulation. Such release is achieved by varying ratios of various components of the transvaginal composition and depends on the chemical and chemico-physical properties of the drug itself. The resulting composition is then incorporated into the device or into a coating of the vaginal device.

The variability of the drugs (analgesics versus anti-migraine or anti-nausea), treatments (dysmenorrhea versus migraine and nausea), targeted delivery sites (uterus versus brain) and formulations (strongly mucoadhesive composition with short transport to uterus with minimized systemic delivery versus delivery of the drug specifically to the systemic circulation) clearly distinguishes two inventions.

Applicants amended claims to be directed solely to anti-migraine and anti-nausea drugs and canceled any other drug that could be used for treatment of dysmenorrhea.

Applicants respectfully submit that the instant claims are not anticipated by Harrison and request that the rejection is withdrawn.

Claims 31-35 are rejected under 35 U.S.C.102(b) as being anticipated by Harrison et al. '327.

Harrison et al. '327 disclose for treatment of dysmenorrhea comprise an intravaginal drug delivery system containing an appropriate pharmaceutical agent incorporated into a pharmaceutically acceptable carrier whereby the pharmaceutical agent is released into the vagina and absorbed through the vaginal mucosa to provide relief of dysmenorrheal (abstract). Harrison et al. '327 further disclose the pharmaceutical agent to comprise aspirin, ibuprofen, ketorolac and naproxen (column 6, lines 30 and 32; claims 3, 10, 13, 16, 18 and 22), and the pharmaceutically acceptable carrier comprises a hydrophilic or hydrophobic carrier, such as semi-synthetic glycerides of saturated fatty acids with 8 to 18 carbons and PEG 6000/1500, respectively (column 6, lines 52-60). Also, Harrison et al., 211. '327 disclose the pharmaceutical formulations further comprising a mucoadhesive agent, preferably hydroxypropyl methylcellulose (column E3, lines 61-67; and claim 6), and a penetration enhancer, preferably ethoxydiglycol (column 7, lines 1-8; and claim 7). Harrison et 211. '327 also disclose the method of applying the pharmaceutical formulation with the aid of an intravaginal delivery device, such as tampon device, vaginal

ring, pessary, tablet, vaginal suppository, vaginal sponge, bioadhesive tablet, bioadhesive microparticle, cream, lotion, foam, ointment, solution and gel (column 7, line 51 through column 12, line 20). Harrison et al. '327 also disclose that preferred formulations for hydrophilic drugs comprise between about 60-90% by weight lipophilic carrier, between about 5- 25% mucoadhesive agent, and between about 5-20% sorption promoter, whereas preferred formulations for lipophilic drugs comprise between about 50-90% by weight hydrophilic carrier, between about 5-20%> mucoadhesive agent, and between about 5- 25% sorption promoter (column 7, lines 9-12 and 22-25; and claim 8).

Applicants disagree. The same arguments as advanced above are appropriate to this rejection. Applicants amended claims to eliminate drugs that are claimed by Harrison. The method for treatment, drugs and the diseases or conditions are different.

The instant claims are not anticipated by Harrison '337. Rejection should be withdrawn. It is so respectfully requested.

Rejections under 35 USC 103

Claims 31-42 and 45 are rejected under 35 U.S.C. 103(a) as being unpatentable over Harrison et al. '909 in view of Penkler et al.

Applicants claim a method for treatment of migraine, migraine headache, nausea, and vomiting associated with chemotherapy, radiotherapy, surgery, pregnancy, pre-menstrual syndrome, menstruation or menopause, with the aid of an intravaginal delivery device comprising administering said intravaginal device and a composition comprising naratriptan, a mucoadhesive agent (i.e. HPMC), a lipophilic or hydrophilic

carrier (mono-, di-, or triglyceride of fatty adds with 8 to 18 carbons or PEG 6000/1500, respectively), and a sorption promoter (ethoxydiglycol).

Examiner argues that Harrison et al. '909 teach a an intravaginal drug delivery system containing an appropriate pharmaceutical agent incorporated into a pharmaceutically acceptable carrier whereby the pharmaceutical agent is released into the vagina and absorbed through the vaginal mucosa, as discussed above.

Examiner admits that Harrison et al. '909 do not teach the pharmaceutical agent to comprise naratriptan. However, Penkler et al. teach naratriptan, sumatriptan and almotriptan as suitable agents for the treatment of migraines (column 7, lines 42-47; column 10, lines 47-48; and claims 1, 8) through vaginal administration (column 13, lines 4-5 and 8).

Therefore, Examiner finds that it would have been prima facie obvious for one skilled in the art at the time of the instant invention to use naratriptan as the pharmaceutical agent in the intravaginal delivery device of Harrison et al. '909, because Penkler et al. teach that naratriptan can be applied via the vagina for treatment of migraines.

Examiner concludes that from the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the

references, especially in the absence of evidence to the contrary.

Applicants disagree. The Harrison reference and its relevance to the instant invention was discussed above. Applicants respectfully submit that Examiner is interpreting th Penkler reference incorrectly and that a combination of the two references do not make the instant invention obvious or prima facie obvious.

Penkler reference teaches pharmaceutical composition for enhancing permeation of basic drugs across the skin, which is a keratinized epithelial barrier, and non-keratinized epithelial barriers, including the nasal, rectal, and vaginal mucosa. Applicants acknowledge that Penkler lists anti-migraine drugs such as naratriptan and sumatriptan as suitable active ingredients for treatment of migraine and also that the drugs are suitable for transdermal or transmucosal delivery, including through vaginal administration (column 13, lines 4-5 and 8), according to Penkler method. However, such delivery is limited by the conditions imposed by Penkler's method.

Examiner makes the rejection to be the prima facie obviousness without considering what properties and conditions are needed for Penkler formulation to achieve the transdermal or transmucosal delivery of the anti-migraine drugs.

The drugs formulated for transdermal or transmucosal delivery disclosed by Penkler are limited to drugs that exist in a positively charged sale form (acid addition salts). Such delivery is only successful when such positively charged drug is in combination with a negatively charged fatty acid or bile acid

(column 1, lines 11-13, column 4, lines 41-43, claims 1, 4 & 6). Therefore, the teaching by Penkler mandates formation of a neutral species by electrostatic attraction between oppositely charged ions that is known to one ordinary skilled in the art as "ion-pairing".

Various applications of successful improvement of transdermal and transepithelial delivery of therapeutic agents using "ion-pairing" have been described in the scientific literature (e.g., "Applications of the ion-pair concept to hydrophilic substances with special emphasis on peptides" by Quintanar-Guerrero et al., (1997), *Pharm. Res.*, 14;119-127 (Copy enclosed for Examiner convenience).

None of those limitations is applicable to the method and composition disclosed in the present invention. The present invention provides a combination of a therapeutically appropriate agent such as an anti-migraine and/or anti-nausea drug with a mucoadhesive composition comprising an inert hydrophilic or lipophilic carrier and a non-ionizable sorption promoter.

Inclusion of the chemically narrowly defined glycol ether or ester as sorption promoter further distinguishes the present invention from Penkler reference that specifically requires incorporation of negatively charged fatty and bile acids as penetration enhancers in order to achieve desired electrostatic interaction for ion-pairing (column 11, lines 8-13).

To compensate for compromised aqueous solubility of the ion-pair resulting from neutralized charge distribution between the counter (positive/negative) ions and the presence of a hydrophobic alkyl chain or rigid sterol moiety of the fatty and

bile acids, respectively, Penkler teaches a composition containing hydrophilic cyclodestrine (column 11, lines 37-51). These natural cyclic oligosaccharides form inclusion complexes with the ion-paired drug and increase membrane permeation of the lipophilic ion-pair by modulating the aqueous boundary layer of the epithelial barrier (see "Effects of cyclodestrins on drug delivery through biological membranes" by Loftsson et al., *J. Pharm. Sci.*, (2007) 96:2532-2546 (Copy enclosed).

In contrast, the instant application does not involve or suggest a method or means to form ion-paired complexes or to increase or enhance aqueous solubility using hydrophilic cyclic oligosaccharides in order to facilitate drug permeation across the vaginal mucosa.

The Applicants wish to emphasize that the same pharmaceutical excipient may serve different roles in different composition despite its identical chemical structure. However, it is relevant to notice and for Examiner to consider that performance functionalities of such excipients critically depend on physicochemical properties of the agent under defined physiological conditions at the site of administration.

The composition of this invention requires the presence of non-ionizable glycol ether/ester derivative acting as penetration enhancers to disrupt important regulatory proteins at cell-cell connections and to perturb the bilayer environment of the vaginal epithelium. As a consequence of these excipient-induced alterations of the physiochemical properties of the biological barrier the incorporated anti-migraine/anti-nausea drug

efficiently permeates across the membrane into the systemic circulation and is able to reach the target site in the brain.

Unique to the vaginal environment is a significantly more acid pH that affects viscosity of mucoadhesive agents. Therefore, the compositions disclosed by the applicant are restricted to limiting mixtures of selected excipients that create, in the physiological environment of the vaginal cavity the optimal physiochemical conditions for the glycol ether/ester derivatives to alter the mucosal barrier properties for improved drug delivery.

Penkler discloses composition using chemically related excipients without restrictions for the tissue it is to be applied to. For example for buccal, sublingual, or nasal administration (column 12, lines 16-43), no regard is given to pH value. As the mucosal pH value of all those claimed sites of administration is significantly greater (pH 6.7-7.5) than in the vaginal cavity, the chemical composition of epithelial cells dramatically varies (e.g., water:protein:lipid ratio). Additionally, and the anatomical barrier consists of only 2-3 cell layers as compared to 20-30 layers as in the vaginal mucosa. For these reasons, a person skilled in the art would recognize that compositions disclosed by Penkler do not comprise suitable conditions for anti-migraine/anti-nausea drugs to efficiently permeate the vaginal mucosa.

Moreover, Penkler supports this conclusion by limiting vaginal administration of the acid addition salt of the water-soluble cyclodextrin inclusion complex to convention pessary

formulations without mucoadhesive agent or sorption promoter (column 12, lines 48-51).

Prima facie obviousness rejection is based on combination of Harrison and Penkler references.

As already discussed above in 102 rejections, Harrison et al., ('327) teaches methods and composition for the treatment of dysmenorrhea. Because the site of administration of those compositions disclosed in this patent is also the vaginal cavity, the list of pharmaceutically acceptable excipients comprises similar inactive carrier materials to prepare conventional vaginal devices, including tampons, suppositories, films, foam, and tablets.

In the assessment of relevant prior art, however, it is essential to distinguish the important physiological change in environmental pH that occurs during menstruation, the time when dysmenorrhea occurs. Normally, the environmental pH value in the vaginal cavity is around pH 4-5 as a consequence of acidic secretions from commensal vaginal microflora. Following the scientific concept of pH partition theory (Penkler, column 1, line 54) the increase in vaginal pH during menstruation from pH value 4-5 to about 7.4 due to the presence of blood can dramatically change the lipophilic properties of an ionizable drug molecule and, consequently, requires different compositions for effective transmucosal drug delivery.

Other relevant changes in the epithelial barrier of the vagina during menstruation include reduced thickness due to altered steroid hormone levels and increased paracellular permeability due to pH-induced modulation of protein-protein

interactions at the tight junctions. As a consequence of those physiological changes, it is not obvious to a person of ordinary skill in the art how to substitute one component with a known function as disclosed by Harrison. Importantly, the therapeutic focus of the disclosed methods and composition in the Harrison patent is unambiguously the uterine muscle (column 1, line 40).

In contrast to the present invention where the composition is designed to maximize transmucosal delivery of drugs into the systemic circulation, the therapeutic advantage of the invention disclosed by Harrison et al., is clearly limited to maximize the deposition of a drug following vaginal administration in the uterine muscle while minimizing systemic drug concentrations that are usually associated with severe side effects (column 5, line 65). For the person of ordinary skill in the art, this clearly separates the two inventions, with Harrison et al. disclosing methods and compositions for local or topical drug delivery, whereas the present invention distinctly centers on methods and compositions suitable for systemic drug delivery.

Taking into consideration all variables required by Penkler and Harrison for achieving the transvaginal drug delivery of the anti-migraine or anti-nausea drug, when this drug, present in its acid additional salt form, would be formulated using the mucoadhesive composition of Harrison directed to targeted drug delivery to the uterus, myometrium or endometrium, such formulation (if the acid addition salt of the anti-migraine drug could be formulated according to Harrison) would achieve at most a targeted delivery of such drug into vagina, uterus, myometrium

or endometrium with minimal, if any, delivery to the systemic circulation.

Such drug delivery would defeat the aim and purpose of the instant invention which is to deliver systemically an efficacious amount of the anti-migraine drug to the brain while avoiding the gastrointestinal system. Additionally and noticeably, Harrison formulations are geared toward delivery during menstruation and if the invention and anti-migraine drugs were delivered during menstruation, the pH conditions of the vagina would further obscure and complicate the transvaginal delivery and would not result in the systemic delivery of these drugs and in treatment of migraine.

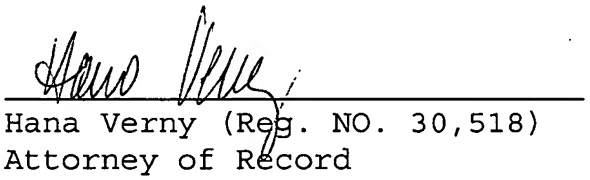
It is respectfully submitted that a combination of Harrison and Penkler references does not make the instant invention obvious and even more so prima facie obvious. The rejection should be withdrawn and the claims passed to issue. It is so respectfully requested.

SUMMARY

In summary, claims 31-50 are pending, claims 31 and 37 are amended and arguments are submitted to overcome Examiner's rejections. With these amendments and arguments, all rejections are overcome. Notice of Allowance is respectfully solicited.

Dated: January 16, 2008

Respectfully submitted,
PETERS VERNY, LLP



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Review

Applications of the Ion-Pair Concept to Hydrophilic Substances with Special Emphasis on Peptides

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Previous investigations have shown that ionic drugs with high aqueous solubilities can be lipophilized by ion-pair formation, with appropriate counter-ions. This type of association may prove promising for several biopharmaceutical, analytical and technological applications. This review examines the ion-pair concept with special emphasis on its application to peptides. The conditions for ion-pair formation of different molecules, as well as their transfer to organic solvents, are described. The use of ion-pairs to increase the permeation of various therapeutic agents, including peptidic drugs, is also discussed. Recent uses of ion-pairs in micro- and nanoencapsulation of peptides are also commented upon.

KEY WORDS: ion-pair; peptides; counter ions; ion-pair extraction; ion-pair enhanced absorption.

INTRODUCTION

Peptides and their analogues are becoming a significant new class of therapeutic agents. This is due to the structural elucidation of numerous natural peptides and the understanding of their role in several physiological processes, the development of systematic methods to produce therapeutic peptides and also to the rapidly expanding field of recombinant DNA technology. Accordingly, the commercial production of peptides for pharmaceutical purposes is now well established and the list of peptides available as therapeutic agents is growing rapidly (1). Unfortunately, peptide drugs can possess chemical and physical properties, including high molecular weight, low partition coefficient, short biological half life, immunogenicity and denaturation, which make them unsuitable for delivery using the conventional absorption routes (2). Among these drawbacks, low lipophilicity is probably the most important factor to overcome. Although the chemical modification of peptides, using natural fatty acids and macromolecules, seems to be appropriate for increasing their lipophilicity, the pharmacological effect of these derivatives is questionable and their enzymatic bioconversion must be confirmed in each case. Furthermore, their formulation and stability have not been studied and like other drug derivatives, they are considered as new drugs by the regulatory agencies and have to be submitted to extended toxicological studies. It is clear that lipophilization of peptides without modification of their chemical structures would be ideal (3,4), not only from the biopharmaceutical point of view, but also from the analytical (e.g. separation and determination in biological samples) and technological (e.g. protein synthesis and incorporation into carriers) standpoints.

Experimental evidence to date suggests that ion-pairing effectively increases lipophilicity of charged drug molecules, both *in vitro* and *in vivo*. The concept has also been applied to peptides in order to obtain more lipophilic drugs that are stabilized via electrostatic interactions (5–7).

This review will focus on the physicochemical parameters involved in ion pair formation with special emphasis on peptidic drugs and will focus on solvent extraction of drugs, and on the enhanced absorption as ion-pairs. Results from experiments carried out in our laboratory involving extraction, permeation and nanoencapsulation of aminoacids and dipeptides as ion-pairs, will also be presented. Analytical aspects such as high-performance ion-pair chromatography for the separation and quantitation of peptides and ion-selective liquid membrane electrodes, as well as the pharmacokinetic and toxicologic features of ion-pair transport across membranes, will not be discussed, since excellent reviews are already available (5,8).

THE ION-PAIR CONCEPT

One of the main problems of the proposed theories for electrolyte solutions has been to account for ion-ion and ion-

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ABBREVIATIONS: a, radius of spherically charged molecule; A^+, cation; A^+,B^-, ion-pair; α, side-reaction coefficient; A(OH), conjugated base of A; B^-, anion; B'^-, anion of a fatty acid; C_L, drug concentration in the luminal phase; C_M, drug concentration in the membrane; C_B, drug concentration in the blood; D, distribution ratio; d, distance; e, electron charge; E_A, degree of extraction of A^+; E, Born energy of charging; ϵ, dielectric constant; k, Boltzmann's constant; K, partition ratio; K_{12}; K_{21}; K_{23}, drug-absorption rate constants for a three-compartment model; $K_{A,Q}$, extraction constant; $K'_{A,Q}$, conditional extraction constant; IPM, isopropyl myristate; q, phase volume ratio; R^+ or R^-, ionizable side chain; S, solvent; T, absolute temperature; V_{org}; V_{aq}, organic and aqueous phase volume; X, adduct; Z_+ and Z_-, charge of the ions.

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solvent interactions. The first satisfactory theory of ionic solutions was proposed by Arrhenius in 1887 (9). He made the bold assumption that electrolytes were completely dissociated into their ions when the solution was infinitely dilute. Arrhenius ascribed the decrease of equivalent conductance, with increasing concentration, to association of the ions into neutral molecules. Arrhenius' basic principles of electrolytic solutions marked a great advance in the electrolyte field and some of them are still valid today. However the application of these principles was limited to solutions of weak acids and bases in water. They failed completely to explain the behaviour of strong electrolytes such as ordinary salts and strong acids and bases where interionic attractive forces cause deviations from the ideal behaviour.

It was not until 1923 that the solution to the electrolyte problem was found by Debye and Hückel (10). Their theory accounted for the thermodynamic properties of electrolyte solutions. They assumed that strong electrolytes were completely dissociated into their ions in aqueous solution and that the deviations of electrolytic solutions, expressed in terms of activities, activity coefficients, and ionic strengths, were due to the electrostatic effects of the oppositely charged ions. However, the Debye-Hückel theory of complete dissociation did not explain the low conductance of strong electrolytes in non-aqueous solutions and it did not extend to include all solvents. In 1926 Bjerrum developed a theory that took into account the interaction of ions at short range (11). He introduced the ion-pair concept and showed how the mass action constant of the equilibrium between ions and ion-pairs was dependent on the dielectric constant of the solvent as well as on the temperature and the size of the ions. The Bjerrum theory was strongly supported by the work of Kraus in the late Thirties and Forties (10,12,13).

Atherton and Weismann (14) investigated the existence of ion-pairs, using electron spin resonance spectroscopy, and demonstrated beyond doubt the association of a sodium cation with a naphthalene radical anion. Ion-pair formation is now an accepted fact, at least in non-aqueous media.

Although ion-pairing phenomena were initially investigated in the field of physical chemistry, the concept was rapidly adopted in the pharmaceutical sciences. In particular, Higuchi and Schill and their co-workers made large contributions and established the basis for its application to molecules of pharmaceutical interest (4,15).

Definition and Nature of Ion-Pairs

Ion-pairs may be defined as neutral species formed by electrostatic attraction between oppositely charged ions in solution, which are often sufficiently lipophilic to dissolve in non-aqueous solvents (7,16). It should be emphasized that the formation of an ion-pair is due only to the so-called outer-sphere interaction and even though this molecular interaction can be written according to the mass action law, no chemical bond of any kind is formed. The general notation A^+, B^- is used to describe an ion-pair product which exists as a stable, thermodynamically distinct species and not as a transient, continuously exchanging association (7,13,15). It is clear therefore that any charged molecule in solution, under certain conditions, can form an ion-pair, with an ion of opposite charge. Thus, as peptides present multiple ionizable sites, depending on their primary structure and the pH of the solution, they are capable of interacting *in vitro* or *in vivo* with appropriate counter-ions. The formation of a peptidic ion-pair results in the "burying" of the charges involved and the alteration of physical properties, for example, lipophilicity (17,18).

Forms of Ion-Pairs

The work of Sadek and Fuoss (19) and that of Winstein *et al.* (20), later confirmed by Roberts and Szwarc (21), showed that an ion-pair can exist in two forms: as a tight or intimate ion pair, or as a loose or solvent separated ion-pair, depending on the nature of the solvent-ion interaction. These authors established that free ions in solution are surrounded by solvent molecules polarized by the electric fields generated by the ionic charges. A sufficiently strong polarization and solvent-ion interaction result in the formation, around each ion, of a tight solvation shell. The presence of such a solvation shell is reflected in the fact that the Stokes radius of the solvated ion is substantially greater than that predicted for the bare ion. An ion possessing a tight solvation shell may approach a counter-ion without hindrance until its solvation shell contacts the partner. Thereafter, either the associate maintains its structure as a loose, solvent-separated ion-pair, or the solvent molecules separating the partners are squeezed out and a tight contact ion-pair is formed. This implies that solvent-separated ion pairs may exist only in those media in which the free ions acquire tight solvation shells; otherwise, only tight contact ion-pairs are produced. It is important to mention that Bjerrum's original concept of a pair of solvated ions that are held together by coulombic attraction, in a solvent of a low dielectric constant, remains valid without modification despite the presence of a solvation shell. For example, if the solvated ion is paired with a bulky counter-ion, the gain in coulombic energy arising from the approach of the partners into close proximity may not be sufficient to accomplish the destruction of the solvation shell. Therefore, such pairs exist only in the loose form.

Solvation of Ion-Pairs

As indicated above, the formation of ion-pairs is only possible if the ions approach each other and reach a critical separation distance (d) given by the Bjerrum's equation:

$$d = |Z+Z-|e^2/2\epsilon kT \qquad (1)$$

where Z+ and Z− are the ionic charges, e is the electron charge, ϵ is the dielectric constant, k is Boltzmann's constant and T is the absolute temperature. The equation shows the importance of the dielectric constant (ϵ) in ion pair formation; accordingly a solvent with a high dielectric constant such as water ($\epsilon = 78.5$) will be unfavourable for ion pair formation, but this does not mean that it is impossible (as we will see later). On the other hand, the interaction becomes increasingly important in solvents with $\epsilon < 40$. Although this rule is applicable to a large number of ion-pair extraction systems (4,15,22), some authors have shown that other non-coulombic contributions can be involved in the ion-association, for example hydrogen bonding, lipophilicity of the ions, and other factors such as the solubility parameter would explain more satisfactorily the solvation of the ion-pair (19,23).

The behaviour of the solvating agent and its affinity for the ion-pair can be explained by the solvation theory proposed

by Higuchi (24). Ion-pairs can be classified, according to the degree of charge accessibility, into three different categories (Fig. 1). In the first case, it is assumed that the cation is large and lipophilic except for the positively charged center. The small external surface would be expected to carry a relatively negative charge per unit area (shown by the external shadow in Fig. 1). This type of system may be effectively solvated by lipophilic molecules having a positively charged surface, e.g. dipolar molecules with acidic protons such as chloroform, phenols and alcohols. Since the bonded solvating molecules would have their polar end buried adjacent to the anion, the appearance presented to the surrounding solvent by the solvated ion-pair would be that of a relatively nonpolar aggregate.

In the second case, the situation is reversed, the ion pair having its cationic charge largely exposed. Solvating species containing nucleophilic sites may be expected to be particularly effective for this type of ion-pair, e.g. ethers, ketones, amides and phosphate esters. The third case is that of an ion-pair with deeply buried charges. Having no exposed electrically unbalanced surface, it would be expected neither to require solvation in order to be readily extractated by nonpolar solvents.

On the other hand, Higuchi attributes ion-pair solubility to the formation of complexes involving association with a discrete number of solvent molecules, which take part in the formation of the ion pair in the organic phase. This solvation can be written as an equilibrium:

$$A^+,B^- + nS \rightleftarrows A^+,B^- \cdot nS \qquad (2)$$

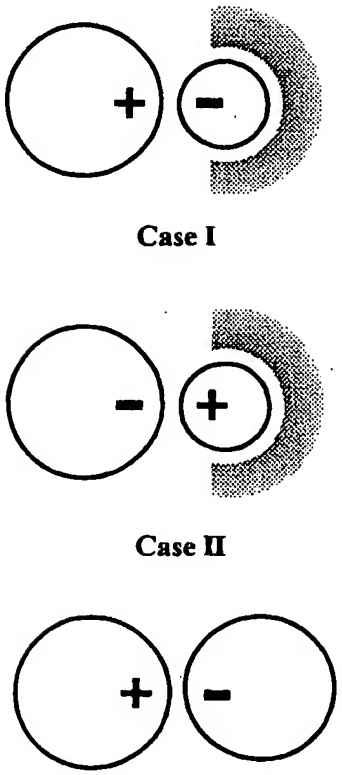

Fig. 1. Classification of ion-pairs according to Higuchi (24). See text.

where A^+ and B^- are oppositely charged ions in solution and nS is the discrete number of solvent molecules assumed to be complexed with the ion-pair. The concept of a specific solvation for ion-pairs has been confirmed using infrared techniques, and nuclear magnetic resonance and electron spin resonance spectroscopies (13).

Ion-Pair in Aqueous Systems

Although ion-pair formation has been considered only for solvents with a low dielectric constant (Bjerrum's ion-pair), the existence of an ion-pair in water or in other highly structured (bonded) solvents is possible when the ions involved are largely hydrophobic. In this case, ion-pairing is due to a solvent mediated effect rather than to an electrostatic interaction (4,7). The term "water structure enforced" ion-pairing was introduced by Diamond (25) in order to explain the existence of ion-pairs in aqueous systems. If both the cation and anion, are large hydrophobic species, the hydrogen-bonded water structure forces them together to maximize the water-water interactions and to minimize the structural perturbation. Water structure enforced ion-pairing involves both electrostatic and hydrophobic interactions, the relative contribution of which is dependent upon both ions' structures and on their immediate environment.

Despite the possibility to form ion-pairs in aqueous solution, the usefulness of this phenomenon is very limited due to the low association constant; furthermore, the ion-pairs exist only at very low concentrations, because of the poor solubility of the ions (4,12,23). An interesting phase diagram for the interactions between large ions in aqueous solution was proposed by Tomlinson (6) for a dianionic drug and a cationic surfactant. This diagram, shown in Fig. 2 reveals that the ion-

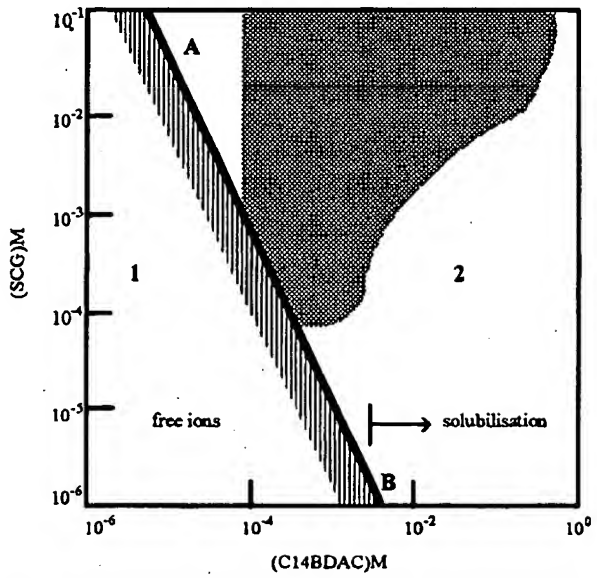

Fig. 2. Phase diagram for the interaction of the dianionic drug sodium cromoglycate (SCG) and the cationic surfactant tetradecyldimethylammonium chloride (C14BDAC). Region 1 has no coacervation and has as its boundary the solubility product line A-B. The shaded area of the visual turbidity region 2 is an area of solubilized coacervate. The dots represent an area of ion pair formation. Adapted from 6.

pair exists only in a narrow range of low concentrations; above this range, colloidal aggregates will be observed.

ION-PAIR EXTRACTION

Probably the most important applications of the ion-pair concept in the pharmaceutical field is the isolation and determination of drug molecules. Ion pair extraction can be described as the extraction of a complex of two oppositely charged ions in an aqueous phase (subscript aq) by a solvent that is not miscible with water (subscript org). This equilibrium can be written as (15):

$$A^+_{aq} + B^-_{aq} \rightleftarrows A^+,B^-_{org} \quad (3)$$

The equilibrium constant of the reaction is normally called the extraction constant of the ion pair, $K_{A,B}$:

$$K_{A,B} = [A^+,B^-]_{org}/[A^+]_{aq}[B^-]_{aq} \quad (4)$$

The magnitude of this constant is dependent upon the nature of the components of the ion-pair and on the properties of the organic solvent.

The distribution ratio, D, of an ion (e.g. for A^+) is defined as:

$$D_A = [A^+,B^-]_{org}/[A^+]_{aq} = K_{A,B} \cdot [B^-]_{aq} \quad (5)$$

The distribution of an ion-pair between an aqueous and an organic phase depends on the equilibrium constant of the ion-pair and on the equilibrium constants of chemical processes (side-reactions) in both phases. Some types of side-reactions are shown in Fig. 3. The side-reactions of A^+,B^- in the organic phase, e.g. dissociation of the ion pair (A^+,B^- to A^+ and B^-) polymerization ($(A^+,B^-)_n$) and adduct-formation with an agent X ($A^+,B^- \cdot X_n$) result in an increase of $K_{A,B}$. On the other hand protolysis of A and B in the aqueous phase and partition result in decrease of $K_{A,B}$. When side-reactions occur, correction coefficients (α) must be introduced to the conditional constant $K'_{A,B}$:

$$K'_{A,B} = K_{A,B}\alpha_{A,B}/\alpha_A \cdot \alpha_B \quad (6)$$

In such cases the extraction constants are calculated by graphical methods (26). It must be emphasized that these side-reactions are not to be considered entirely in a negative light since they can improve both selectivity and the degree of extraction.

The degree of extraction (E) of A^+ (as A^+,B^-) into the organic phase with a single extraction can be calculated from:

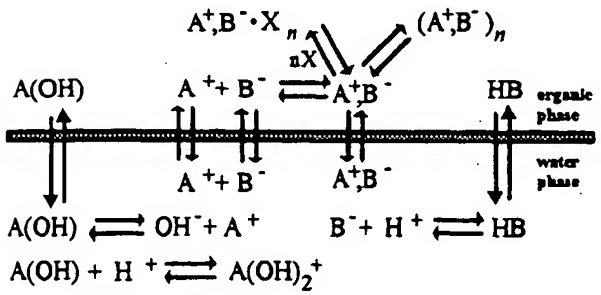

Fig. 3. Schematic representation of side-reactions involved in the extraction of an ion-pair in the two-phase system. Adapted from (15).

$$E_A(\%) = 100D_A \cdot q(1 + qD_A)^{-1} \quad (7)$$

where q is the phase volume ratio V_{org}/V_{aq}.

The extraction of a drug can be regulated by the nature and the concentration of the counter-ion as well as by the nature of the organic solvent. It should be noted that the solvent is a component which takes part in the formation of the ion-pair, and that its selection will influence the degree of extraction. In most studies chlorinated solvents such as chloroform and dichloromethane are used as the organic phase. These solvents exhibit low dielectric constants and are hydrogen donors, which makes them particularly suitable for the extraction of several ion-pairs. Furthermore, their tendency to extract anions as acids is limited, and the incidence of other side-reactions such as dissociation and polymerization of the ion-pairs is low. When poor extraction is observed with chlorinated solvents, due to the high hydrophilicity of ion-pair components, the addition of more polar solvents (e.g., alcohols) which form adducts with the ion-pair in the organic phase is recommended (27).

Extraction of Peptides as Ion-Pairs

Considering that peptides are ampholytes with very low partition ratios, it is to be expected that their extraction as ion-pairs, with solvents of low dielectric constants, will be limited; however, more polar solvents should give better extractions. Experiments made in our laboratory seem to confirm this idea. Tables 1 and 2 show some extraction constants with different solvents for tryptophan (Trp) and tryptophan-leucine (Trp-Leu), respectively. The ion-pair partition was made with the species in cationic form, due to the low extraction achieved in previous tests at the isoelectric point. Chlorides, nitrates and sulphates were used as counter-ions, using the corresponding acids at 0.1 N in aqueous solution. It is clear that an increase in solvent dielectric constant (ϵ) significantly improves the extraction. For propylene carbonate and butanol, the log K value clearly increases with the hydrophobicity of the counter-ion in the aqueous phase at pH 1, $NO_3^- > Cl^- > SO_4^{-2}$ (28,29). The dif-

Table 1. Effects of Inorganic Counter-Anions on the Logarithm of the Partition Ratio (log K) of Tryptophan Using Different Solvents at pH 1

Solvent (dielectric constant; hydrogen bonding capability)	log K (±CV[a])		
	SO_4^{2-}[b]	Cl^-[b,c]	NO_3^-[b,c]
Chloroform (4.8; poor)	b. d. l.[d]	b. d. l.	b. d. l.
Dichloromethane (9.1; poor)	−3.46 (0.42)	−1.66 (0.93)	−0.77 (0.65)
Ethyl acetate (6.0; moderate)	−0.04 (1.46)	−1.57 (0.47)	−1.46 (0.40)
1-Butanol (17.1; strong)	−0.68 (1.46)	0.01 (0.39)	0.26 (2.32)
Propylene carbonate (65.1; moderate)	−0.18 (0.73)	−0.12 (2.0)	0.47 (0.49)

[a] CV: Coefficient of variation (%) (n=4).
[b] The hydrophobicity of anions decreased as $NO_3^- > Cl^- > SO_4^{2-}$ (28).
[c] The log of distribution constant of HNO_3 and HCl between propylene carbonate and water is −1.05 and −1.60, respectively (29).
[d] b. d. l. : below detection limit.

Table 2. Effects of Inorganic Counter-Anions on the Logarithm of the Partition Ratio (log K) of Tryptophan-Leucine Using Different Solvents at pH 1

Solvent (dielectric constant; hydrogen bonding capability)	log K (±CV[a])		
	SO_4^{2-}[b,c]	Cl^-[b,c]	NO_3^-[b,c]
Chloroform (4.8; poor)	b. d. l.[d]	b. d. l.	b. d. l.
Dichloromethane (9.1; poor)	−2.03 (0.43)	−1.64 (0.63)	−2.23 (0.93)
Ethyl acetate (6.0; moderate)	−1.05 (1.01)	−0.92 (0.65)	−0.94 (3.12)
1-Butanol (17.1; strong)	0.25 (2.31)	0.88 (2.33)	1.13 (3.21)
Propylene carbonate (65.1; moderate)	0.48 (2.15)	0.58 (2.11)	1.07 (1.2)

[a] CV: Coefficient of variation (%) (n=4).
[b] The hydrophobicity of anions decreased as $NO_3^->Cl^->SO_4^{2-}$ (28).
[c] The log of distribution constant of HNO_3 and HCl between propylene carbonate and water is −1.05 and −1.60, respectively (29).
[d] b. d. l. : below detection limit.

ferences in the extracted quantity of Trp and Trp-Leu in butanol and propylene carbonate are attributed to their hydrogen-bonding capacity.

Akamatsu *et al.* (17), studying the partition of peptides in octanol/water systems (Table 3), found somewhat surprisingly that a considerable increase in the partition coefficient was obtained using inorganic ions in the aqueous phase at pH 1; in the same way, these authors observed that the degree of extraction depended on the hydrophobicity of the counter ions. These data suggest that for the same counter-ion, the degree of extraction of Trp dipeptides depended on the relative hydrophobicity of the attached amino acid: Trp>Phe>Leu>Ala. Accordingly, the hydrophobicity scales for amino acid side-chains (30) could be useful for the prediction of the degree of extraction.

A recent interesting contribution dealing with the ion-pair extraction of a therapeutic peptide has been made by Adjei *et al.* (18). They evaluated the partition of leuprolide acetate, a nanopeptide with multiple ionizable sites, in octanol/water systems at different pH values and using different counter-ions. The results showed that partition is increased by the presence of alkyl sulfonic acids, depending on the length of the alkyl chain and that the increase in lipophilicity of leuprolide ion-pairs may be proportional to the extent of ionization of the imidazolyl nitrogen of histidine.

Table 3. Effects of Counter-Anions on the Logarithm of the 1-Octanol/water Partition Ratio (log K) for Some Dipeptides at pH 1 (from reference 17)

Peptide	log K (pH 1)		
	ClO_4^-[a]	NO_3^-[a]	Cl^-[a]
Trp-Trp[b]	1.10	0.50	0.21
Trp-Phe[b]	0.77	0.17	0.02
Trp-Leu[b]	0.68	0.08	−0.18
Trp-Ala[b]	−0.68	−1.22	−1.42
Phe-Phe	0.37	0.17	0.43
Leu-Phe	0.14	−0.44	−0.66

[a] The hydrophobicity of anions decreases as $ClO_4^->NO_3^->Cl^-$. While the free energy of ClO_4^-, NO_3^-, Cl^- ions from water to nitrobenzene is 8.7, 24.4 and 30.5 kJ/mol; the log of the distribution constant of $HClO_4$, HNO_3 and HCl between propylene carbonate and water is −0.05, −1.05 and −1.60, respectively (29).
[b] Relative hydrophobicity of amino acid side chains.

An illustrative analytical development using ion-pair extraction for peptide isolation and analysis was presented by Uvashkiv (31), who developed a spectrophotometric method for the determination of cyclic octapeptidic antibiotics during fermentation. The method is based on the extraction of antibiotic from alkaline broth with butanol. Ion-pairs formed between the octapeptides and bromothymol blue were extracted into chloroform from a solution buffered to pH 7.5. It is important to point out that the extraction of these peptides by chloroform as ion-pairs was possible because of their exceptionally low water solubility.

Although there are only a few publications concerning ion-pair extraction of peptides, two common themes seem to be evident: the transfer of peptides from aqueous to organic solution is improved by using polar solvents and by decreasing, or avoiding, the presence of ionized groups which do not take part in ion-pair formation (ion suppression). The possibility of transferring peptide intermediates and protected peptides to organic solvents, could have interesting implications for peptide and protein synthesis (32,33).

ION-PAIRING AS A SYSTEM FOR ABSORPTION ENHANCEMENT

Biological membranes can be considered as lipoidal barriers, and thus ionized molecules cannot generally cross them due to unfavourable partitioning (7,34,35). However, ionized solutes like paraquat, suxamethonium, phenothiazines and some quaternary ammonium compounds, are readily absorbed from the gut. Shanker (35) proposed that one of the probable mechanisms for the penetration of organic ions through the gastrointestinal barrier was by formation of a less polar complex with some material normally present in the lumen. Passage of an ionized drug through a membrane (ionophoresis) must involve either the existence of an aqueous pore, or the transport of the ion from an aqueous environment to a lipid environment, then across the lipid (lipoprotein) and finally transfer into the aqueous phase on the other side of the membrane. This hypothesis proposes that the ion-ionophore interaction provides a mechanism for drug absorption. One way of visualizing ion-pair transport is in terms of the difference in the level of specific resistance encountered by ions and ion-pairs for permeation through membranes. Thus, an ionized drug could be considered as a spherically charged molecule of radius with an energy of charging determined by the dielectric constant of the medium (ϵ), according to the expression $E = e^2/2\epsilon a$, where E represents the Born energy of charging. Due to the inverse dependence of energy on ϵ, large energy differences exist between low and high dielectric media. This produces a great energy barrier for ion partitioning and flux into hydrophobic membranes. The formation of ion-pairs might lower E by "burial" of the ion charge by the counter-ion and thus the transport of ionic drugs through hydrophobic membranes would be enhanced (4).

Irwin *et al.* (16) were the first to test the ion-pair hypothesis for the lipophilization of an ionic drug (isopropamide) using

an exogenous counter-ion (trichloroacetate). The results of this study indicated that the rate and efficiency of gastrointestinal absorption of isopropamide were increased by ion-pair formation with trichloroacetate. The authors stated that it might be possible through selection of appropriate ion-pair formers to improve the efficiency and uniformity of absorption of highly ionized drugs from the gastrointestinal tract. This hypothesis received support from subsequent work investigating the permeation of different hydrophilic ionizable drugs through artificial and biological membranes *in vitro*, *in situ* and *in vivo*, *ex vivo* experiments (4,16,34,36,37). It is important to point out that absorption experiments with ion-pairs have not always pointed at clear-cut mechanisms. In those cases, an increase of drug absorption was not evident in the presence of a counter-ion, or the increase of the absorbed amount was not attributed to ion-pair formation, but to a direct effect of the counter-ion: mucosal binding, mucosal erosion, interfacial tension lowering effect on the gut wall, increased surface activity of the drug, etc. (38,39). Thus, additional experiments using neutral molecules such as caffeine, which is unable to form ion-pairs, should be carried out in order to distinguish whether the enhancement is due to ion-pair formation or to the disruptive effect of the counter ion on membrane integrity (40).

The increase of drug permeability by ion-pair formation can be generally attributed to an increase in the partition coefficient factor. Suzuki *et al.* (36) studied the partition of quinine for its absorption across rat rectal mucosa, using a three compartment model of drug absorption:

$$C_l \underset{K_{21}}{\overset{K_{12}}{\rightleftarrows}} C_m \xrightarrow{K_{23}} C_b \qquad (8)$$

where C_l, C_m and C_b represent the drug concentration in the luminal phase, membrane and blood, respectively, and K_{12}, K_{21}, and K_{23} are rate constants. The results showed an evident increase of K_{12} caused by the presence of the counter-ion. The authors stated that this may be caused by the increase of drug concentration in the absorptive membrane surface which is related not only to the physicochemical properties of the ion-pair systems in the medium, but also to the chemical nature of the surface of the absorptive site. This could explain why the permeation of certain drugs was unaffected by ion-pair transport. However, the physicochemical basis of the failure of these drugs to undergo ion-pair transport remained unclear and ion-pair enhancement of transport *in vivo* remains controversial (5).

In the Seventies *in vitro* studies used artificial membranes (37) as models of biological membranes for evaluating the transport of drugs as ion-pairs. The model membranes provided improved control and interpretation of the variables involved and made interpretation of results easier (4,34). In general, those studies were performed using a two-compartment diffusion cell, with a lipophilic membrane, porous or not, that could be impregnated with a liquid representing the biological barrier (41). The donor compartment was a buffer solution with a pH which favoured the formation of the ion-pair, or a non-toxic non-aqueous medium (i.e. propylene glycol) in which the ion-pair was formed (4).

An interesting variation on these studies, was the incorporation into the membrane of a counter-ion as a carrier. Hadgraft *et al.* (42) showed that ionic drug transport across an artificial lipid membrane impregnated with isopropyl myristate (IPM) could be increased by incorporation of a counter-ion capable of forming ion-pair into the membrane. A pH gradient was used to facilitate the transport of the ions from an aqueous compartment across a non-polar organic phase to an aqueous receptor against the ion concentration gradient. This principle was developed in an attempt to facilitate the transport of ionic drugs across the stratum corneum. Green *et al.* (34) showed that a pH-gradient made it possible to increase the *in vitro* flux of naphazoline across human cadaver skin previously pretreated with fatty acids (Fig. 4).

In general, the mechanism of penetration through synthetic membranes is assumed to involve a partitioning between the aqueous phases and, further, a diffusion through the film. Ågren *et al.* (37), studying the permeation of a quaternary compound through nylon membranes with perchlorate as the counter-ion, suggested that transfer was taking place mainly by partitioning and diffusion and, to a much lesser extent by penetration through pores. On the other hand, Lee and Kim (4) proposed that the permeation of ion-paired drugs in non-aqueous systems through 2-hydroxyethyl methacrylate/styrene membrane could proceed via both the partition and the pore mechanisms. This conclusion was founded on permeation experiments based on the free volume theory (22,42) to identify the mechanism of solute diffusion through membranes, the permeation being thus dependent on the molecular radius of the drug.

Ion-Pairing to Enhance Peptide Absorption

The use of absorption enhancers as a means of making peptide administration more facile has been extensively studied (2,42) The results have shown that these can indeed promote peptide absorption. One of the first papers, by Nishihata *et al.* (43), studied the disappearance of phenylalanine and its analogues by perfusion across rat rectal tissue, enhanced by the presence of salicylate or 5-methoxysalicylate. The effect of these adjuvants was attributed to the alteration of the membrane barrier, increasing its permeability; however, the simultaneous absorption of the adjuvant was also required. Data from Okada

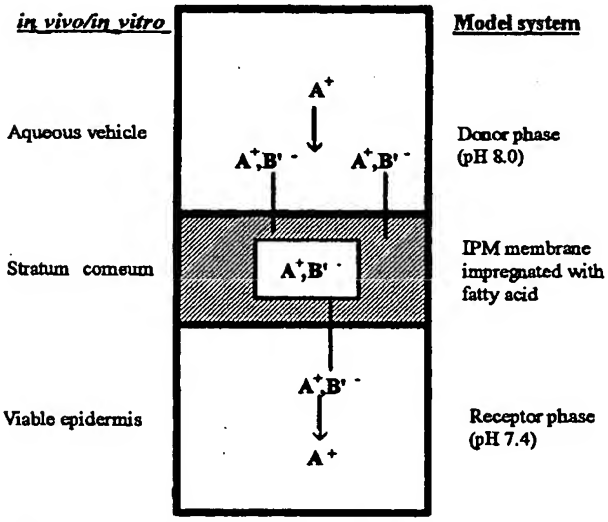

Fig. 4. Conditions required to facilitate the transport of a cation (A^+) from an aqueous donor phase across an IPM membrane impregnated with fatty acid (B'^-) to an aqueous receptor phase. Adapted from (32).

et al. (44) showed that vaginal absorption of leuprolide and insulin could be increased by the use of organic acids. The mechanism was attributed to the acidification and chelating ability of the acids. Studies on the nasal absorption of insulin in the presence of surfactants (45) have suggested that the promoting effect of bile acid salts was due to their direct effect on the nasal mucosa and their inhibitory effect on proteolytic enzymes. It is evident from these investigations that the mechanisms of action of the peptide absorption enhancers are not clearly understood and different hypotheses can be made to explain them. Lee (46) established that ion-pair formation between the enhancer and the peptide drug was a probable way to affect the thermodynamic activity of the peptide and its permeability. Zhou and Po (47) have produced arguments in favour of this idea. They reevaluated the effect of cholate and its analogues on the nasal absorption of insulin, stating that these compounds might enhance the absorption of peptide and protein drugs by binding to insulin. This binding may protect against hydrolysis too, because this would prevent the formation of an enzyme-insulin complex which aligns the catalytic site on the protease. Other facts that would support this assumption are the tendency of cholate and its analogues to form ion-pairs and their low activity as aminopeptidase inhibitors (38).

Green *et al.* (48) showed that it is possible to enhance the flux of peptides across the skin using conventional enhancers for ion-pairing peptides, in order to mask their ionic characteristics thereby increasing their solubility in the stratum corneum. [D-Ala^2]-methionine-enkephalinamide was examined as a model peptide since it possessed only one NH_2 group which was significantly ionized over the pH range of interest and therefore had the potential to pair with oleate anions. The carboxyl residue was blocked by amide formation. Results showed an evident increase of the *in vitro* permeation of peptide across human skin, due to the presence of oleic acid.

Ganem *et al.* (49) in our laboratory found that the *in vitro* permeation of a dipeptide (Trp-Leu) through the palatal mucosa of pig was increased using saline hydroalcoholic solutions. In the absence of sodium chloride, lower permeation was observed. The results suggested that the effects of conformational changes or possible denaturation of keratin in the stratum corneum or the lipid extraction, caused by ethanolic systems, were insufficient to allow the passage of the peptide. The increased permeability was attributed to the probable ion-pair formation between sodium chloride and the peptide.

APPLICATION OF ION-PAIR FORMATION TO THE MICROENCAPSULATION OF PEPTIDES

Micro- and nanospheres are promising candidates as carriers for oral, parenteral and other routes of administration of peptidic drugs. However, one of the main problems of these techniques is the poor entrapment of water-soluble drugs, principally with those that involve an o/w emulsification, due to partition of the drug from the organic phase into the continuous aqueous phase. Yamakawa *et al.* (50,51) studied the influence of different fatty acid salts contained in the external phase on the encapsulated amount of an hexapeptide (neurotensin) in poly(D,L-lactic acid) microspheres, by an o/w solvent evaporation technique. The results indicated a considerable increase of the entrapment ratio in the presence of fatty acid salts. The authors stated that the effect of fatty acid salts was due to partitioning into the oily phase at the solvent-water interface. The coexistence of neurotensin and fatty acid salts as ion pairs in the oily phase was effective in entrapping neurotensin into the microspheres.

Recently, Niwa *et al.* (52) showed that the leakage of nafarelin acetate during the preparation of nanospheres of poly(D,L-lactic-co-glycolic acid) by the nanoprecipitation method can be partially avoided by the addition of a small amount of negatively charged phospholipid such as dipalmitoyl phosphatidylglycerol or dicetyl phosphate. The presence of these additives in the organic phase (acetone) decreased the total leakage of peptide into the aqueous phase during polymer precipitation. The proposed mechanism involved ion-pair formation between the amine groups of nafarelin (histidyl or arginine residues) and the negatively charged phospholipids.

The experiments made in our laboratory to encapsulate a dipeptide (Trp-Leu) in poly(D,L-lactic acid) nanospheres by the emulsification-diffusion method (53), using the peptide in the form of ion-pair in propylene carbonate (Table 2), indicated a low encapsulation efficiency (<3%). This result was attributed to the leakage of the peptide contained in the globules into the aqueous phase, during solvent diffusion. Apparently, the velocity of ion-pair dissociation was faster than polymer precipitation in the interfacial region, where the transport of solvent to the continuous phase was effected.

CONCLUSIONS

It has been shown that ion-pair formation allows the lipophilization of hydrophilic molecules and ions. This kind of association has been widely used for the separation and determination of molecules of pharmaceutical interest. In this respect, it is important to emphasize that the routine separation of peptides with reverse-phase high-performance liquid chromatography is based on ion-pair formation essentially with trifluoroacetic acid, using increasing concentrations of acetonitrile.

Although the usefulness of ion-pair systems to promote permeation of drugs through membranes is controversial, and in some cases questionable, the data presented in this review suggest that ion-pairing should be considered as a useful method for increasing the bioavailability of drugs and enhancing permeation, mainly when the administration is made by routes where the physicochemical equilibrium is not rapidly undermined by the surrounding medium. Therefore, an understanding of the correlation between the physicochemical properties of ion-pairs and their interaction with membranes is necessary.

The application of ion-pair formation to peptides opens interesting perspectives. The concept could provide a useful approach to solve the problems of limited peptide absorption by non-parenteral routes, and a peptidic ion-pair might contribute to the development of formulations with optimized absorption. The transfer of peptides as ion-pairs into solvents could also have applications in the synthesis of proteins and in the development of methods for their micro- and nanoencapsulation. Clearly, further investigations are necessary to identify the possible applications of peptide-based ion-pairs.

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B

Effects of Cyclodextrins on Drug Delivery Through Biological Membranes

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ABSTRACT: Cyclodextrins have proven themselves to be useful functional excipients. Cyclodextrin derivatives can be hydrophilic or relatively lipophilic based on their substitution and these properties can give insight into their ability to act as permeability enhancers. Lipophilic cyclodextrins such as the methylated derivatives are thought to increase drug flux by altering barrier properties of the membrane through component extraction or fluidization. The hydrophilic cyclodextrin family also modulate drug flux through membranes but via different mechanisms. The current effort seeks to provide various explanations for these observations based on interactions of hydrophilic cyclodextrins with the unstirred water layer that separates the bulk media from biological membranes such as the gastric mucosa, cornea and reproductive tract. Theories on the serial nature of resistances to drug flux are used to explain why hydrophilic cyclodextrins can enhance drug uptake in some situation (i.e., for lipophilic material) but not in others. In addition, the nature of secondary equilibria and competition between cyclodextrins and rheologically important biopolymers such as mucin are assessed to give a complete picture of the effect of these starch derivatives. This information can be useful not only in understanding the actions of cyclodextrin but also in expanding their application and uses. © 2007 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 96:2532–2546, 2007

Keywords: cyclodextrin; drug delivery; unstirred water layer; water structure; permeation; membrane

INTRODUCTION

Cyclodextrins (CDs) are natural cyclic oligosaccharides that are formed through enzymatic degradation of starch.[1] The three most common CDs, αCD, βCD and γCD, are composed of six, seven and eight α(1→4)-linked α-D-glucopyranose units, respectively, with a hydrophilic outer surface and a somewhat lipophilic central cavity (Table 1). The hydroxyl functions are orientated to the exterior whereas the central cavity is lined by skeletal carbons and ethereal oxygens, which give it a lipophilic character. Although the naturally occurring CDs and their complexes are hydrophilic, their aqueous solubility is rather limited, especially that of βCD. Random substitution of the hydroxy groups located on the outer surface of the CD molecule, even by hydrophobic moieties such as methoxy functions, results in dramatic improvement in their solubility (Table 1). In an aqueous environment, CDs form inclusion complexes with many lipophilic drug molecules through a process in which water molecules

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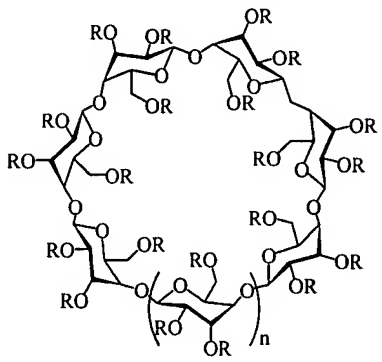
Table 1. Some CDs That can be Found in Marketed Pharmaceutical Products



Cyclodextrin	n	R = H or	Subst.[a]	MW[b] (Da)	Solubility in Water[c] (mg/ml)
α-Cyclodextrin (αCD)	0	–H	0	972	145
β-Cyclodextrin (βCD)	1	–H	0	1135	18.5
2-Hydroxypropyl-β-cyclodextrin (HPβCD)	1	$-CH_2CHOHCH_3$	0.65	1400	> 600
Sulfobutylether β-cyclodextrin sodium salt (SBEβCD)	1	$-(CH_2)_4SO_3^-\ Na^+$	0.9	2163	> 500
Randomly methylated β-cyclodextrin (RMβCD)	1	$-CH_3$	1.8	1312	> 500
γ-Cyclodextrin (γCD)	2	–H	0	1297	232
2-Hydroxypropyl-γ-cyclodextrin (HPγCD)	2	$-CH_2CHOHCH_3$	0.6	1576	> 500

[a]Average number of substituents per glucose repeat unit.
[b]MW: Molecular weight.
[c]Solubility in pure water at approx. 25°C.

located inside the central cavity are replaced by some lipophilic structure of the drug molecule. Recent studies have shown that CDs also form non-inclusion complexes as well as complex aggregates.[1–3] Non-covalent bonds are involved in complex formation and, in aqueous solutions, drug molecules bound in the complex are in rapid equilibrium with free solubilized drug molecules.[4] Worldwide about 30 different pharmaceutical products containing CDs have reached the market.[1,5,6] CDs have mainly been used as complexing agents to increase aqueous solubility of poorly soluble drugs, to increase their oral bioavailability and chemical stability.

The chemical structure of CDs is associated with a large number of hydrogen bond donors and acceptors, a high molecular weight (from 972 to over 2000 Da) and a low octanol/water partition coefficient (Log $P_{o/w}$ from less than −3 to almost 0) predicting that they do not readily penetrate biological membranes.[7] In fact, experimental evidence has shown that only negligible amounts of hydrophilic CDs (Log $P_{o/w} < -3$) penetrate lipophilic biological membranes such as skin and the gastrointestinal mucosa.[8–12] Only the free drug is able to permeate lipophilic membranes[13–15] and excess CD, more than is needed to solubilize the drug in the aqueous exterior, in fact reduces drug penetration through membranes (Table 2). However, there is one exception: lipophilic CDs, such as randomly methylated β-cyclodextrin (RMβCD, Log $P_{o/w}$ −1.2), reduces the barrier function in certain cases and thereby enhance drug delivery through biological membranes such as the nasal mucosa.[16]

In this article, we review the effects of hydrophilic CDs on drug permeation through membranes and discuss the possible mechanism of action based on the current knowledge of the structural characteristics of water and the unstirred water layer (UWL) juxtaposed to the membrane of interest, also referred to as the aqueous diffusion layer, static diffusion layer or stagnant liquid film.

STRUCTURAL CHARACTERISTICS OF WATER

Although the chemical structure of water (H_2O, MW 18 Da) appears trivial, its physicochemical

Table 2. Some Observations Regarding Cyclodextrins (CDs) and Drug Bioavailability or Penetration Through Biological Membranes

Observation	Reference
CDs can only act as enhancers from an aqueous exterior	13,68,74
Hydrophilic CDs reduces the drug release from w/o creams but enhances the release from o/w creams. When applied to excised human skin CDs enhance drug delivery from o/w creams through the skin	68,69
Only negligible amounts of hydrophilic CDs and CD complexes are able to penetrate biological membranes such as skin and gastrointestinal mucosa	8–10,12,13
CDs do not, in general, enhance permeability of hydrophilic water-soluble drugs through lipophilic biological membranes	60,75–77
CDs are, under certain conditions, able to extract lipophilic components from biomembranes such as stratum corneum but pretreatment of the membranes with hydrophilic CDs does not usually enhance permeability. Reduced permeability is commonly observed at relatively high CD concentrations	74,78–83
CDs can enhance drug bioavailability through stabilization of drug molecules	84–86
Due to their very low bioavailability hydrophilic CDs are, in general, considered non-toxic when given orally	11
CDs and conventional penetration enhancers, like fatty acids, or mechanical enhancers, like iontophoresis, can have additive or synergistic effect on drug delivery through biological membranes	78,87–91
Number of studies using various biomembranes, and under several different experimental conditions, have shown that excess CD, i.e. more than needed to solubilize a given lipophilic drug in an aqueous vehicle, results in decreased drug penetration through the membrane. Maximal enhancement is obtained when just enough CD is used to solubilize the lipophilic drug in the aqueous vehicle	60,81,90,92–101
In oral drug delivery, greatest bioavailability enhancements are, in general, obtained for Class II (high permeability, low solubility) drugs. No or even decreased bioavailability are obtained for Class I (high permeability, high solubility) and Class III (high solubility; low permeability) drugs	5

properties are far from simple.[17–20] Both the melting point of water (MP 0°C) and its boiling point (BP 100°C) are much higher than expected when compared to other group VIA hydrides in the periodic table such as hydrogen sulfide (H_2S; MW 34 Da, MP −85°C, BP −60°C), hydrogen selenide (H_2Se; MW 81 Da, MP −66°C, BP −41°C) and hydrogen telluride (H_2Te; MW 130 Da, MP −49°C, BP −2°C).[21] The unusually high hydrogen bond strength which acts to hold water molecules together result in enhanced cohesion and, consequently, in an elevated BP. The electronegativity (EN) of the heavier atoms: S (EN 2.6), Se (EN 2.6) and Te (EN 2.1), are much lower than that of oxygen (EN 3.4), and close to that of hydrogen (EN 2.2). Thus, the hydrides of the heavier atoms are unable to form hydrogen bonds.[22] For the same reason other physicochemical properties of liquid water, such as its dielectric constant (ε 78.5 at 25°C), density (1.000 g/ml at 3.98°C), surface tension and heat of vaporization (40.65 kJ/mol), are higher than expected although many of the anomalies of water are still not well understood.[21,23] The dielectric properties of organic solvents, for example glycerol (ε 42.5 at 25°C), ethanol (ε 24.3 at 25°C), isopropanol (ε 18.3 at 25°C) and diethyl ether (ε 4.3 at 20°C), are much lower than that of water as a result of much weaker intermolecular bonding and solvent cohesion. In liquid water, hydrogen bonding leads to "cooperative bonding" where water molecules link together to form water clusters ($(H_2O)_n$).[24] This means that formation of one hydrogen bond makes it easier to form an even stronger second hydrogen bond. Hydrogen bonds are quite strong (20–40 kJ/mol) compared to van der Waals interactions (about 1.3 kJ/mol) but much weaker than covalent bonds (about 400 kJ/mol). Water can be thought of as a gel structure where extensive connectivity of different regions is established by hydrogen bonds.[25] The local structure within different regions of bulk water

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is continuously changing leading to homogeneous density, even at the nanoscale level.[20,23] However, co-solvents, solutes and solid surfaces will all effect the water structure. For example, ethanol–water binary mixtures have microscopic phase separation at the cluster level.[26] Structure-breaking solutes (chaotropes) destroy the hydrogen-bonded water network in a manner which is similar to the effect of increased temperature while structure-forming solutes (kosmotropes) increase the structural complexity. Sugars, such as fructose, glucose and sucrose, behave as chaotropes at low concentrations, while at higher concentrations they act as kosmotropes.[27] In aqueous solutions, water molecules form hydrogen bonds with hydroxy groups on the CD surface forming a hydration shell around the dissolved CD molecule.[28–31] At aqueous concentrations below about 35% (w/v), CDs exert only small effects on viscosity but these effects can be significant at higher concentrations.[32] This indicates that CDs behave like sugars, as chaotropes at low concentrations but as kosmotropes at high concentrations. Water-soluble polymers, such as cellulose derivatives and polyethylene glycols, form hydrogen bonds with water that are stronger than water-water bonds, i.e. a positive hydration characterized by low exchange rate of the water molecules around the polymers, resulting in increased viscosity even at concentrations below 1% (w/v).[33,34]

Water structures at membrane surfaces are strongly affected by the ability of the surface to form hydrogen bonds with water.[35] Virtually no water is adsorbed to graphitized carbon, a hydrophobic surface. The self-association of water molecules is much stronger than interactions of water molecules with the hydrophobic surface, a phenomenon analogous to the hydrophobic effect observed when non-polar solutes are dissolved in water.[20,36–38] Whereas water in contact with hydrophilic surfaces, e.g. some silica-based materials, forms a water film where hydrogen bonding in the network of water molecules are partly substituted by bonds between the water molecules and the surface.[36] These interactions result in reduction of the mobility of water molecules directly adsorbed on the surface by more than one order of magnitude. The water layer is only a couple of water molecules thick.[20] At cell membranes, water molecules are bound to phospholipids, proteins and other membrane constituents resulting in a water layer thickness of about 1 nm.[39] Water molecules are bound to the skin surface as well as within the outermost layer of the skin, the stratum corneum.[40] Mucosal epithelium (mucosa) contain mucosal cells that secretes mucus, a gel-like fluid containing mainly water (~95%) and mucin.[41] Mucins are large glycoproteins with MW ranging from 0.5 to 20 MDa. Some are membrane bound but others are not. The viscous mucus forms a relatively thick (up to about 100 μm) UWL in, for example, the gastrointestinal tract, the respiratory tract, the ocular-rhino-otolaryngeal tracts and the reproductive tract.[42] Under unstirred *in vitro* condition the UWL can be significantly thicker, even in absence of mucus.[43–46]

Formation of water clusters, hydrophobic interactions and water structures at membrane surfaces can have profound implications on the biological effects of drugs, including their ability to permeate various membrane barriers.[47]

THEORETICAL BACKGROUND

Higuchi described drug permeation through multilayer barriers as series of additive resistances analogous to electric circuits.[48] Later Flynn and Yalkowsky described mathematically drug permeation through lipophilic membrane sandwiched between UWLs.[49,50] They based their work on earlier observations by Zwolinski and coworkers emphasizing that the UWL must be treated as part of the total barrier.[51] Assuming independent and additive resistances of the individual layers, the total resistance (R_T) of a simple membrane (Fig. 1) can be defined as:

$$R_T = R_D + R_M + R_R \tag{1}$$

where R_D, R_M and R_R are the resistances in the UWL at the donor side, within the membrane and in the UWL at the receptor side, respectively. Since the permeability constants are the reciprocals of the resistances the following equation is obtained:

$$J = P_T \cdot C_V = (R_D + R_M + R_R)^{-1} \cdot C_V = \left(\frac{1}{P_D} + \frac{1}{P_M} + \frac{1}{P_R}\right)^{-1} \cdot C_V \tag{2}$$

where J is the flux of the drug through the membrane, P_T is the overall permeability coefficient, C_V is the concentration of the compound in the vehicle (i.e. donor phase), and P_D, P_M and P_R are the permeability coefficients in the UWL at

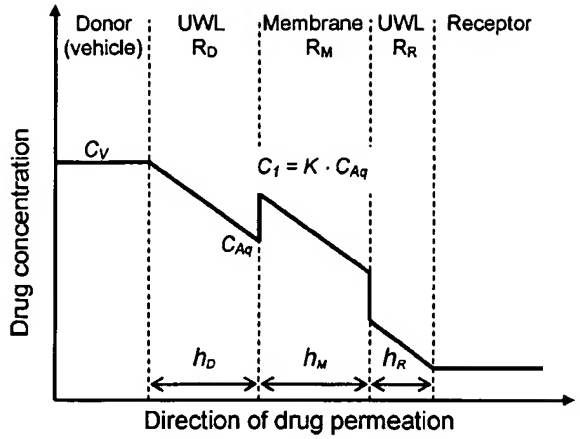

Figure 1. Schematic drawing of drug permeation from a donor phase through UWL on the donor side, through a membrane, and finally through UWL on the receptor side. C_V: drug concentration in the donor (vehicle); C_{Aq}: drug concentration in the UWL immediate to the membrane surface; C_1: drug concentration within the membrane at the donor side; K: the drug partition coefficient between UWL and the membrane; h_D: thickness of the UWL on the donor side; h_M: thickness of the membrane; h_R: thickness of the UWL on the receptor side. R_D, R_M and R_R are the resistances in the UWL at the donor side, within the membrane and in the UWL at the receptor side, respectively.

the donor side, within the membrane and in the UWL at the receptor side, respectively. If R_R is assumed to be negligible due to relatively rapid removal of drug molecules from the receptor side of the membrane, Eq. 2 is obtained:

$$J = \left(\frac{P_D \cdot P_M}{P_D + P_M}\right) \cdot C_V \tag{3}$$

If the value of P_M is much greater than the value of P_D then Eq. 3 becomes:

$$J = \left(\frac{P_D \cdot P_M}{P_D + P_M}\right) \cdot C_V \approx \left(\frac{P_D \cdot P_M}{P_M}\right) \cdot C_V = P_D \cdot C_V \tag{4}$$

and the UWL becomes the main barrier, i.e. permeation is diffusion controlled. On the other hand, if P_D is much greater than P_M then Eq. 5 is obtained:

$$J = \left(\frac{P_D \cdot P_M}{P_D + P_M}\right) \cdot C_V \approx \left(\frac{P_D \cdot P_M}{P_D}\right) \cdot C_V = P_M \cdot C_V \tag{5}$$

and permeation will be membrane controlled. The relationship between the permeation coefficient (P) and the diffusion coefficient (D) is given by Eq. 6:

$$P = \frac{D \cdot K}{h} \tag{6}$$

where h is the thickness (h_D, h_M or h_R in Fig. 1) and K is the partition coefficient between the aqueous phase and the membrane. For P_D and P_R the value of K is unity. Finally D can be estimated from the Stokes–Einstein equation:

$$D \approx \frac{R \cdot T}{6\pi \cdot \eta \cdot r \cdot N} \tag{7}$$

where R is the molar gas constant, T is the absolute temperature, η is the apparent viscosity within the UWL or the lipophilic membrane, r is the radius of the permeating drug molecule and N is Avogadro's number. Thus, the diffusion constant within the UWL (D_D) will decrease with increasing viscosity of the layer as well as with increasing molecular weight of the drug. For example, small lipophilic drug molecules frequently possess a large permeability coefficient through the lipophilic membrane (i.e. large P_M value) and, thus, may be able to permeate lipophilic membrane much faster that they can be transported through the UWL. Under such conditions, diffusion through the UWL becomes the rate-limiting step in the absorption process.

Many biological membranes have an aqueous mucus layer adjacent to the membrane surface. These gel-like structures with fairly high viscosity are relatively effective diffusion barriers (Eqs. 6 and 7). Hydrophilic membrane surfaces will also lead to formation of structured water layers at the membrane surface (Table 3). Under such conditions the UWL can have a significant contribution to the overall membrane barrier, i.e. $P_M \approx P_D$ or $P_M > P_D$ (Eqs. 3 and 4) or in other words if $R_M \approx R_D$ or $R_M < R_D$ (Eqs. 1 and 2).

CYCLODEXTRINS AS PENETRATION ENHANCERS

In general, chemical penetration enhancers, such as fatty acids, ethanol, surfactants, bile salts, Azone® and chitosan, not only enhance transdermal drug delivery but also intranasal, ocular and buccal drug delivery as well as intestinal drug absorption.[52–59] These enhancers increase drug permeation through the membranes by penetrating the membranes and decreasing their barrier properties, making the membranes more

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Table 3. Membrane Properties and the Effect of CD Solubilization on Drug flux Through the Membrane.[a]

Membrane	Structure	Surface Characteristics	UWL (h_D) (μm)	R_D/R_M[b] Ratio	Effect of Increasing CD Conc.[c]		Reference
					Drug Saturated	Drug Unsaturated	
Artificial membranes							
Semi-permeable cellophane	Porous symmetric regenerated cellulose membrane with MWCO 500 Da	Hydrophilic with hydrogen bonding OH-groups	Membrane thickness	—	No effect	Decreasing permeability	67,75,102
Semi-permeable cellophane	Porous symmetric regenerated cellulose membrane with MWCO 12–14,000 Da	Hydrophilic with hydrogen bonding OH-groups	Membrane thickness (200 μm)	—	Increasing permeability	Some decrease in permeability	15,67,75,99,102
Cellophane-octanol membrane	Porous symmetric regenerated cellulose membrane (MWCO 12–14,000 Da) with fused octanol/ nitrocellulose matrix membrane	Hydrophilic with hydrogen bonding OH-groups	210	>1	Increasing permeability	Decreasing permeability	15,103
PAMPA	Dodecane solution of 1,2-dioleoyl-sn-glycero-3-phosphocholine on a porous microfilter disc	Somewhat hydrophilic and able to form hydrogen bonds	>1000	>1	Increasing permeability	Decreasing permeability	45,46,104
Cell cultures							
Caco-2	Human colon carcinoma cells grown on semipermeable supports	Somewhat hydrophilic and able to form hydrogen bonds	>1000	>1	Increasing permeability	Some decrease in permeability	43,44,96
Skin							60,81,91

Table 3. (*Continued*)

Membrane	Structure	Surface Characteristics	UWL (h_D) (μm)	R_D/R_M[b] Ratio	Effect of Increasing CD Conc.[c]		Reference
					Drug Saturated	Drug Unsaturated	
Hairless mouse skin	Lipophilic membrane (stratum corneum), stirring of donor phase	The epidermis is about 40 μm thick with a somewhat hydrophilic surface		<1	No effect	Decreasing permeability	105,106
Hairless mouse skin	Lipophilic membrane (stratum corneum), no stirring of donor phase	The epidermis is about 40 μm thick with a somewhat hydrophilic surface	>1	>1	Increasing permeability	Decreasing permeability	60,67,75,105
Human skin	Lipophilic membrane (relatively thick (~12 μm) stratum corneum)	The epidermis is about 90 μm thick with a somewhat hydrophilic surface	>1	<1	No effect	Decreasing permeability	101,105,107
Epithelium (in humans)							
Eye cornea/sclera	Collagen and elastic fibers (sclera) or flat epithelium cells with tight junctions in the intercellular space (cornea)	Mucus/tear fluid	~8 (*in vivo*)	>1	Increasing permeability	Decreasing permeability	92,97,98,108,109
Nasal mucosa	Partly ciliated epithelium covered with mucus	Mucus	~50	>1	Increasing permeability	Decreasing permeability	94
Buccal mucosa	Saliva over keratinized and nonkeratinized mucosa	Saliva	70–100 (*in vivo*)	>1	Increasing permeability	Decreasing permeability	108,110
Intestinal mucosa	Surface gel layer (or mucus) over villi and microvilli.	Mucus	30–100 (*in vivo*)	>1	Increasing permeability	Decreasing permeability	42,64,108,111
Lung mucosa	Surface gel layer over a periciliary layer	Mucus	10–15 (*in vivo*)	<1	Increasing permeability	No or little effect	64,108

Based on permeation of somewhat lipophilic drugs (MW <700 DA) that are able to form hydrophilic CD complexes. No stirring of donor phase, unless otherwise indicated, but stirring of the receptor phase ($h_R \approx 0$; Eq. 2).

[a]The effects of hydrophilic CDs. Lipophilic CDs, such as the methylated βCDs, are able to penetrate into lipophilic membranes and change their barrier properties.

[b]The resistance in the UWL on the donor side (R_D) and the membrane (R_M) is a function of both the thickness (h_D or h_M) and the viscosity (η) of the barriers, as well as binding of the permeating molecules to, for example, mucin in the UWL.

[c]The effect will depend on the relative resistance of the membrane barriers (R_D/R_M).

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permeable by, for example increasing their hydration, modifying their intracellular lipid domains or enhancing drug partition into the membranes by changing their solvent nature. These chemical penetration enhancers enhance membrane permeation of both hydrophilic and lipophilic drugs, both from non-aqueous and aqueous donor phases. Hydrophilic CDs, such as 2-hydroxypropyl-β-cyclodextrin (HPβCD) and sulfobutylether β-cyclodextrin sodium salt (SBEβCD), are on the other hand (Table 2):

(a) Unable to permeate biological membranes such as skin and gastrointestinal mucosa to any significant extent.
(b) Unable to enhance drug permeation from lipophilic environments.
(c) Unable to enhance permeation of hydrophilic drugs.
(d) Able to enhance permeation of lipophilic drugs.
(e) Able to reduce drug permeation through lipophilic membranes by decreasing drug partition from the exterior into the membrane.
(f) Able to increase the chemical stability of drugs at the aqueous membrane exterior.

Thus, for CDs the potential mechanism of action must be different from those of the chemical penetration enhancers.

The Chemical Potential

The chemical potential of drug in the donor phase (i.e. the vehicle or UWL on the donor side in Fig. 1) will also affect drug permeability through the membrane.[48] If the barrier function of a membrane is unaffected by the vehicle composition, the permeability will increase with increasing chemical potential. The value of the partition coefficient (K in Eq. 6) is at its maximum when the chemical potential of the drug in the donor phase is maximized thereby optimizing the tendency of the drug molecule to leave the donor phase and enter the membrane. The chemical potential of a given lipophilic drug in aqueous CD solution saturated with the drug is constant and does not increase with increasing CD concentration. Since only the free drug and not the drug/CD complex is able to partition into the membrane, the observed increase in drug flux with increasing CD concentration[15,60] cannot be explained by an increase in the chemical potential. Furthermore, excess CD, more than is needed to solubilize the drug in the aqueous donor phase, will decrease the chemical potential of the drug and that leads to a decrease in the partition coefficient and consequently a decrease in drug permeation.[61]

Physical Enhancers

Physical enhancers have been used to enhance topical drug delivery, e.g. through the skin or into the eye, in a controllable manner. For example, a small electrical current can be applied to enhance membrane penetration of ionized drugs. The wave energy of ultrasound decreases the barrier potential of biological membranes and in electroporation aqueous pathways are created in lipid bilayer membranes.[62] These physical methods can be applied to increase permeation of both lipophilic and hydrophilic drugs, and for general drug molecules of diverse physicochemical properties and sizes. The permeation enhancing properties of CDs are quite different from those of the physical enhancement.

The UWL

The existence of an UWL during drug diffusion through membranes and drug dissolution is well established.[63] However, the contribution of an UWL to the overall barrier function of a membrane is frequently ignored.[44] The thickness of the UWL can be significant (Table 3) and mucus and other surface structures can enhance its barrier function by increasing its viscosity (η) that leads to an overall decrease in the diffusion coefficient (D in Eq. 7). Studies have shown that drug diffusion through mucus is up to 100-times slower than through pure water.[64] It is possible that CD complexation of lipophilic, and somewhat water-insoluble, drug molecules enhances drug diffusion through the UWL. Increase in the concentration gradient over the UWL will increase the rate of drug diffusion through the layer (Fig. 1). Drug permeation through the UWL depends also on the UWL thickness, decreasing with increasing thickness (h in Eq. 6). Under *in vitro* conditions, the thickness of the UWL can be well above 1000 μm in the unstirred aqueous donor phase and *in vivo* its thickness is frequently 10–100 μm. However, the thickness of the UWL depends also on the physicochemical properties of the permeating drug molecule, including their ability to form

ionic and hydrogen bonds with mucin, and thus fixed UWL thickness for all drugs does not exist.[65]

The Membrane Barriers

The effects of hydrophilic CDs on drug flux through various types of artificial and biological membranes are summarized in Table 3 together with a brief description of the membrane characteristics, including the approximate thickness of the UWL on the donor side and the relative resistance of the UWL (R_D) and the membrane itself (R_M). The resistance is a function of the thickness of the barriers (UWL or the membrane barrier) and their viscosity, increasing with increasing thickness or increasing viscosity (Eqs. 6 and 7). The resistance will also increase with increasing ability of the permeating molecules to bind to membrane constituents such as water, mucin and collagen.

Intestinal Drug Absorption

It has been shown that for intestinal absorption, the main step in the absorption process is diffusion through the stagnant mucus layer (UWL), together with transfer across the mucus/membrane interface.[66] Studies of the relationship between gastrointestinal drug absorption and the biopharmaceutics classification system (BCS) have shown that CDs enhance the bioavailability of Class II drugs that have low aqueous solubility but show good absorption from solutions (low solubility, high permeability). On the other hand CDs do not enhance bioavailability of Class III drugs (high solubility, low permeability).[5] Class III drugs are hydrophilic and do not, in general, form inclusion complexes with CDs. Furthermore, CD complexation of Class III drugs can reduce their ability to partition from the aqueous exterior into the lipophilic membrane (i.e. the CD complexation will lower their K-value in Eq. 6).

Transdermal Drug Delivery

Skin is much less permeable than epithelium and since it does not contain aqueous gel structures like mucus, its UWL is usually much thinner. Thus, under normal conditions CDs do not enhance drug delivery through skin. However, under *in vitro* conditions using hairless mouse skin, which is more permeable than human skin, and when the aqueous donor phase is unstirred, the permeation resistance in the UWL (R_D) can be sufficient to make $R_D \geq R_M$. Under such conditions CDs can enhance dermal and transdermal drug delivery (Table 3).[67] Also, CDs enhance dermal and transdermal delivery of lipophilic drugs from oil-in-water (o/w) creams where the UWL is extended into the cream layer on the skin surface.[68,69] Again, the CD complexation can reduce the ability of lipophilic compounds to partition from the exterior into the skin barrier, a property that sometimes is used to prevent absorption of, for example, sun screeners into and through skin.

Caco-2

The barrier properties of Caco-2 membranes are rate-limited (i.e. possess a relatively low R_M) and under such conditions the adjacent UWL on the donor side has significant contribution to the overall barrier function (Table 3). In the Caco-2 model the aqueous donor phase is usually unstirred resulting in relatively thick UWL and consequently to $R_D > R_M$. Under such conditions hydrophilic CDs can enhance delivery of lipophilic drugs through the membrane.

PAMPA and other artificial membranes

In a previous study we have shown that in the PAMPA system, the thickness of the UWL and its contribution to the overall membrane barrier depends on the stirring rate.[46] In absence of HPβCD, drug permeability increased with decreasing UWL thickness to a certain minimum values of about 40 μm. Addition of HPβCD to systems exhibiting UWL thicknesses greater than 40 μm significantly increased the drug flux through PAMPA. The effect of HPβCD appeared also to be related to stability constant (K) of the drug/CD complex with flux increasing with increasing K value.[46] This suggests that hydrophilic CDs enhance flux when the UWL resistance (R_D) has significant contribution to the overall barrier resistance. CDs are able to enhance drug delivery through artificial membranes if the experimental conditions are such that $R_D \geq R_M$. Table 3 shows that CDs can, in general, enhance drug permeation through membranes if the permeation resistance of the UWL is about equal or greater than the resistance of membrane barrier (i.e. when $R_D \geq R_M$ or $P_M \geq P_D$, Eq. 3 and Fig. 1), but how do CDs enhance the permeation?

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CDs AND UWLs

Excess CD in the UWL will decrease drug partition from the UWL into the membrane (i.e. the value of K in Eq. 6) which explains why excess CD decreases drug permeation through the membranes (Table 3).[61,70] However, since only the free drug is able to permeate the lipophilic membranes, and not the CD molecules or drug/CD complexes, it is difficult to explain why CDs enhance drug delivery through membranes from drug saturated CD solutions, especially since the concentration of free drug does not increase with increasing CD concentration. In aqueous CD solutions saturated with drug, only the concentration of drug/CD complex increases with increasing CD concentration.[60] Since CDs only enhance permeation of relatively lipophilic drugs from aqueous donor phases (vehicles), their enhancing effect must be associated with the physicochemical properties of water and the drug/CD complexation. In aqueous solutions, the cooperative hydrogen bonding leads to formation of water clusters that can decrease mobility of dissolved drug molecules. At membrane surfaces, the cooperative hydrogen bonding leads to formation of a relatively thin ($\ll$1 μm) UWL where mobility of the water molecules is significantly reduced. Water-soluble polymers, like mucin, can increase the thickness of this layer to 100 μm (*in vivo*) or even more (>1000 μm) under some *in vitro* conditions. These relatively thick and viscous UWLs, where mobility of dissolved molecules is severely limited compared to pure aqueous solutions, can induce permeation resistance (R_D) that is greater than the permeation resistance of the membrane itself (R_M) (see Table 3). Interactions of the permeating drug molecules with, for example, mucin will result in even further increase in R_D. Recently it was shown that by eliminating hydrophobic interactions between mucin fibers and hydrophobic surfaces resulted in enhanced particle permeation and that large nano-particles can permeate faster through mucosa than smaller particles.[71] The CD molecules are three to four times larger than the drug molecules and, thus, should permeate UWL at a slower rate than the drug molecules (Eq. 7) but since CDs behave as chaotropes at CD concentrations used in the donor phases (vehicles), the effect should be less than expected based on their MW. Also, it should be remembered that the rates for formation and dissociation of drug/CD complexes are very close to the diffusion controlled limits and that drug/cyclodextrin complexes are continuously being formed and broken down.[4] Based on extensive review of the literature, it appears that CDs enhance drug delivery through the UWL by one or more of the following mechanisms:

(a) CD complexation increases the total amount of dissolved drug molecules in the aqueous donor phase. This increases the concentration gradient of the drug over the UWL leading to more rapid drug delivery to the membrane surface. Since drug release from the CD complex is more rapid than the flux of drug molecules through the UWL, this will increase the availability of free drug molecules immediate to the lipophilic membrane surface.
(b) Since low MW carbohydrates behave as chaotropes at relatively low concentrations, CDs will disrupt the hydrogen-bonded water network in the UWL. This could facilitate penetration of drug/CD complexes through the UWL and increase drug availability at the membrane surface. However, there are no indications that CDs enhance permeation of free drug molecules through the UWL.
(c) CD complexation of drug molecules could possibly prevent them from interacting with molecules in the UWL, such as mucin, increasing their overall delivery rate to the membrane surface.

It is possible that CDs may have other additional mechanisms of action that have not yet been elucidated. Also it is still not clear how other excipients in the aqueous donor phase, such as water-soluble polymers, can enhance drug delivery from aqueous CD solutions.[72,73]

CONCLUSION

It is clear that hydrophilic CDs can only enhance drug delivery when the permeation resistance of the UWL on the donor side is about equal or greater than the resistance of membrane barrier. This is frequently the case when drugs permeate mucosal epithelium where mucosa forms the UWL. Hydrophilic CDs do not enhance drug delivery through membranes if the lipophilic membrane barrier is the main permeation barrier. When aqueous vehicles, such as hydrogels and o/w

creams, are applied to membranes, the UWL is extended into the vehicle and under such conditions CDs can increase drug delivery from the vehicle through the membrane.

CDs only enhance permeation of drug molecules that are able to permeate given membrane with relative ease once the dissolved drug molecules are in contact with the membrane surface, for example absorption of BCS Class II drugs from the gastrointestinal tract. In the case of lipophilic membranes, the greatest enhancement is usually obtained with relatively small, lipophilic drug molecules. Finally, CDs are only able to enhance permeation of drugs that readily form CD complexes.

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